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Acute toxicity of synthetic pyrethroid cypermethrin (25% EC) on histology of liver in *ophiocephalus striatus*

AV Dorlikar[1], AS Mohite[2]
[1, 2] P.G. Department of Zoology, Sevadal Mahila Mahavidyalaya, Nagpur, Maharashtra, India

Abstract
Cypermethrin is a synthetic pyrethroid, which is widely used against various agricultural, veterinary, horticultural and household pests. In the present investigation, an attempt was made to assess the acute toxic effect of sublethal concentration of Cypermethrin (25%EC) on the liver of *Ophiocephalus striatus*. The 96 hours LC_{50} dose of Cypermethrin (25% EC) was found to be (0.72 ± 0.007µl lit $^{-1}$). Liver was dissected out to observe the histopathological changes in the liver after exposure to Cypermethrin. Significant changes were noted in the liver. Study revealed the degeneration of the liver cells, hypervacuolation, pyknotic nuclei, loss and degeneration of connective tissues. Nuclear hyperplasia and hypertrophy was noted in the liver.

Keywords: *Ophiocephalus striatus*, liver, acute toxicity, Cypermethrin, LC_{50}

1. Introduction
Use of pesticides becomes an essential part of the intensive agriculture. Increased use of various pesticides to protect the crop and increase the yield of the crop is posing a great threat to the aquatic ecosystem. A synthetic pyrethroid is an organic compound similar to the natural pyrethrins produced by the flowers of pyrethrums (*Chrysanthemum cinerariaefolium* and *C. coccineum*). Pyrethroids are least acutely toxic to mammals due to poor absorption and rapid biotransformation. Natural pyrethroids are chemically unstable and thus they break down rapidly upon exposure to air and sunlight. Pyrethroids act on GABA receptors in the synapse and cause the neurotoxicity in organisms [1]. Pyrethroids causes destruction of neurotransmitters and inhibition of acetylcholine in the fishes [2, 3].
In aquatic media, a toxin come in direct contact through gills and rapidly enter the blood stream of the fishes and causes the histopathologial alterations in the vital organs of the body like gills, liver etc. and also causes the physiological stress on the aquatic organisms. Exposure of insecticide causes the respiratory distress in the fishes and hampers the respiratory rate and impairs the metabolism in the fish. Poisoning in fish weakens the fish or lead to the death of the aquatic organisms like fishes and molluscs. Thus, in the present study, an attempt has been made to note the acute toxicity of Cypermethrin and observe the histopathological changes in the liver of *Ophiocephalus striatus* after exposure to sublethal dose (LC_{50}) of Cypermethrin (25%EC) for 96 hours.

2. Materials and Methods
For experiment purpose, *Ophiocephalus striatus* (average length 15 ± 1.5 cm, average weight 100 ± 14.75 g) were procured from the local fisherman of Sakkardara Market, Nagpur in well oxygenated polythene bags without injury. The pesticide used for present experiment was Cypermethrin 25% (EC) Shoot, supplied by Yawalkar Agro Industries Corporation Limited, Nagpur. Acute toxicity bioassays were performed in 30 litre glass aquaria, having 20 litres of unchlorinated water. Fishes were acclimatized for seven days prior to experiment. No mortality of the fishes was observed during the acclimatization. Different bioassay sets having different concentrations of Cypermethrin (25% EC) were arranged. Three replicas were performed for each concentration. The observations were made up to 96 hours. Dead fishes were promptly removed from the aquaria. Feeding was stopped before 24 hours of the commencement of the experiment. LC_{50}, 95% confidance limits, goodness of fit were estimated by probit analysis method of Finney (1971) [4]. Paraaldehyde solution was used for anaesthetizing the fishes. Fishes were sacrificed after 96 hours from each control and Cypermethrin treated group. Liver was dissected out, cleaned in physiological saline solution and fixed in Bouin's fluid for 24 hrs. Dehydration was done and tissues were cleared in a xylene and embedded in the paraffin wax. Blocks were prepared. Tissues were cut at 5-6 microns thickness with the help of Rocking microtome. The ribbon of the sections was spreaded on glass slides which is already having Mayer's albumin. Humason Haematoxylene-Eosine double staining technique was followed for the staining [5].

3. Results
Histology of Liver in Control *Ophiocephalus striatus*
In fish, liver is a relatively large organ and lighter brown in colour. In liver of control *Ophiocephalus striatus*, hepatocytes appeared as a continuous mass forming compact distinct cords or lobules. Hepatocytes were round or polygonal in shape containing granular cytoplasm, centrally situated prominent spherical nucleus with dark nucleolus and having homogeneous parenchyma. The blood capillaries in the hepatocytes were hepatic sinusoids. Sinusoidal capillaries were irregular, narrow, thin walled and appeared throughout the stroma. In the lumen of the sinusoids, erythrocytes and macrophages were present. Large cells resting on the surface of the sinusoid endothelium are known as kupffer cells. Kupffer cells were found among the sinusoidal endothelium, which were small

and few in number. The centre-lobular veins and Melanomacrophage centres (MMCs) were supported by thin connective tissue. The cytoplasm was densely stained around the nucleus and slightly stained towards the periphery of the cell wall (Figs. 1, 2, 3).

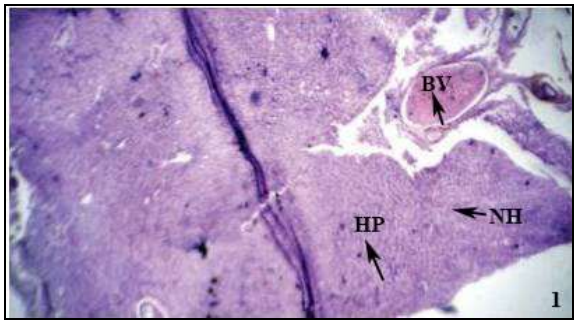

Fig 1: Section of liver of control fish, *Ophiocephalus striatus* showing normal hepatocytes, normal blood vessels and homogenous parenchyma. (H & E × 100).

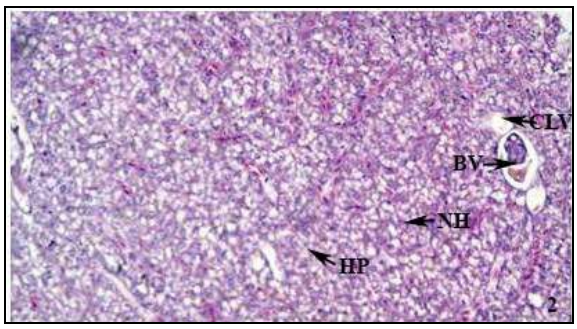

Fig 2: Histological section of liver of *Ophiocepalus striatus* showing homogenous parenchyma, normal blood vessels and normal hepatocytes (H & E × 400).

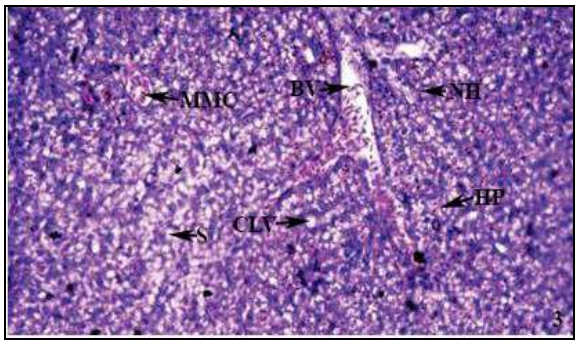

Fig 3: Histological section of liver of *Ophiocepalus striatus* showing homogenous parenchyma, normal blood vessels and normal hepatocytes (H & E × 400).

Abbreviations: NH- Normal hepatocytes, HP- Homogenous parenchyma, CLV- Centrolobular vein, S- Sinusoid, MMC- Melano macrophase centre, BV- Blood vessel

Histology of Liver in *Ophiocephalus striatus* exposed to Cypermethrin (25%EC):

Histopathological investigations of liver exposed to Cypermethrin (25%EC) revealed histological abnormalities in the liver. There was partial disruption of normal chordal arrangements of the hepatocytes. Histological alterations were, degeneration in the hepatocytes, focal areas of necrosis, aggregations of inflammatory cells between the hepatocytes and damage of blood vessels. Change in the shape of hepatocytes occured. These became polygonal to rounded in shape due to hyper-vacuolation which in turn results into complete disintegration of hepatocytes. Disintegration of hepatocytes causes pycnotic nuclei. (Fig. 4). Nucleus of hepatocytes became irregular in shape. Nuclear hypertrophy and nuclear vacuolation was prominent. Cytoplasmic degeneration, and eosinophillic granules were noted in the cytoplasm. Nuclear degeneration, necrosis of hepatocytes, damage of blood vessels with hemorrhage and melano-macrophage centres were observed. Necrosis of interstitial parenchymatous tissues was noted. Pyknotic nuclei were distributed randomly throughout the liver due to necrosis causing extensive damage of liver cells. Distended sinusoidal spaces were observed which were filled with edematous fluid having eosinophillic material (Figs. 5, 6).

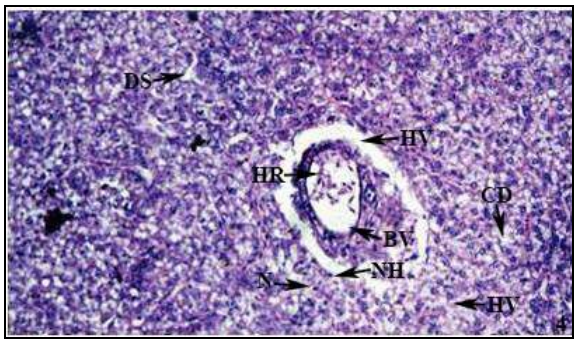

Fig 4: Histological section of liver of *Ophiocepalus striatus* showing highly vacuolated hepatocytes (hyper vacuolation) hetergenous parenchyma, haemorrhage in blood vessels, cytoplasmic degeneration and necrosis of hepatocyte. (H & E × 400).

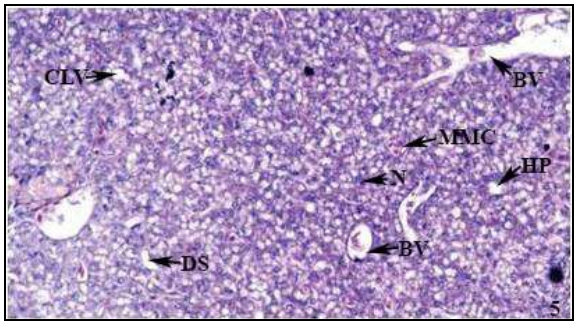

Fig 5: Histological section of liver of *Ophiocepalus striatus* showing highly Vacuolated hepatocyte (hyper vacuolation) hetergenous parenchyma, haemorrhage in blood vessels, cytoplasmic degeneration and necrosis of hepatocyte. (H & E × 400).

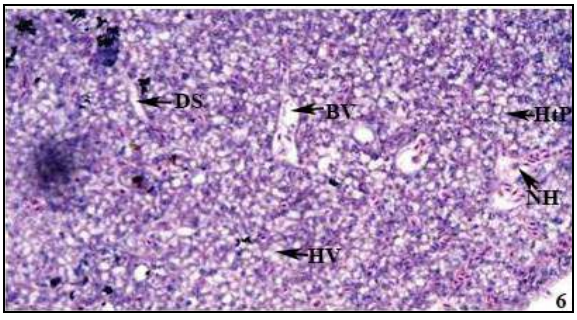

Fig 6: Histological section of liver of *Ophiocepalus striatus* showing hyper vacuolation of hepatocytes, hetrogenous parenchyma, cytoplasmic degeneration and complete necrosis of hepatocytes.

Abbreviations: BV- Blood vessel, HV- Hypervacuolation, DS- Dilation of sinusoid, MMC- Melanomacrophase centre, N- Necrosis, NH- Neclear hyperthrophy, CD- Cytoplasmic degeneration, HR- Hemorrhage, HtP- Heterogenous parenchyma, CLV- Centrolobular vein

4. Discussion

Principle function of liver is the storage of nutrients and secretion of digestive enzymes [6]. It also plays vital role in complex enzymatic processes such as accumulation and biotransformation of xenobiotics in the fish [7]. Histology of Liver in fish *Ophiocepalus striatus* exposed to Cypermethrin for 96 hours revealed the significant changes in the histology of the liver tissue. Pyknosis and extensive vacuolation in the hepatocyte nucleus causes the disruption of the anabolic function of the liver and alters the anabolic function of the liver. Synthesis of many enzymes which are essential for different metabolic pathways get hampered which affects the functioning of the vital organs of the fish and causes the death of the fish. Many researchers have noted the pyknotic hepatocyte nucleus in different species of the fish exposed to various types of contaminants in the water [8, 9, 10]. Pyknosis may be responsible for loss of cellular integrity and necrosis in liver tissue [11, 12, 13]. Cytoplasmic vacuolation that is hyper-vacuolation was also observed during the present study following the exposure of fish to the Cypermethrin. Similar observations were noted by the Velisek *et al*. [14] in *Oncorhynchus mykiss* after exposure to bifenthrin and Hadi and Alwan [15] in *Tilapia zillii* after exposure to aluminum. According to Hinton and Lauren [16] loss of protein synthetic activity and aggregation of microtubules leads to the vacuolization of hepatocytes. Necrosis of the hepatic cells was observed as a result of Cypermethrin toxicity. Olufayo and Alade [17] had studied the effect of Cypermethrin on *Heterobranchus bidorsalis* and noted the necrosis of hepatocytes as fish become unable to regenerate the hepatocytes. Prabhahar *et al*. [18] and Devi and Mishra [19] have also observed the necrosis in liver cells of *Channa punctatus* and *Cirrhinus mrigala* after exposure of chlorpyrifos and cadmium compound respectively. The melano-macrophage centres are clusters of phagocytic cells and the elements of the immune system of fish [20]. Macrophages plays important role in scavenging, phagocytosis and antigen processing [21, 20, 22]. MMCs plays important function of detoxification and destruction of endogenous and exogenous xenobiotics [23, 20, 24]. In teleosts, these MMCs are mainly concentrated in the head kidney, liver and spleen [24, 25, 26, 27, 28]. These MMCs may be playing important role of detoxification.

Damage of blood vessels, distended sinusoidal spaces, hypertrophy of nucleus was also noticed in the liver of fish exposed to Cypermethrin in the present study. Many workers have studied the histopathological changes in liver of various fishes [29, 30, 11, 31, 32, 13]. The change in the shape of the hepatocyte from polygonal to rounded and vacuolated may be due to osmotic imbalance and stress induced by Cypermethrin exposure.

5. Conclusion

The present study indicates Cypermethrin (25% EC) causes the significant alteration in the normal histology of the liver and thus affects all the physiological functions related to the liver. Smaller doses of this pesticide have great potential to cause the death of the fishes and aquatic organism and causes the disturbance in the delicate balance of aquatic ecosystem. Death of the fishes may cause the economic loss of the fisheries.

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